REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources,
gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of :

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	26 Dec 2001	FINAL REPORT 1 July 98 - 31 Dec 2000

4. TITLE AND SUBTITLE
The Optical Patch Clamp Stage III

5. FUNDING NUMBERS
N00014-98-1-0703

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Arlington, VA 22217

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

This award has resulted in 3 major achievements: 1) Second harmonic imaging microscopy (SHIM), a new modality for non-linear optical microscopy. SHIM, has been developed and shown to enable 3D imaging of thick specimens with sub-micrometer resolution. 2) Enhancement of second harmonic generation (SHG) signals by gold nanoparticles has been demonstrated and shows great potential for functional mapping of biological surfaces at the single molecule level. 3) A series of new dyes have been synthesized that have uniquely advantageous properties for both SHG and protein labeling applications.

20011231 102

14. SUBJECT TERMS
optics, dyes, sensors, nanoparticle, second harmonic generation imaging, microscopy

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

FINAL REPORT

GRANT NUMBER: N00014-98-1-0703

PRINCIPAL INVESTIGATOR: Leslie M. Loew (les@volt.uchc.edu)

INSTITUTION: University of Connecticut Health Center

GRANT TITLE: The Optical Patch Clamp. Stage III. Single Channel Activity by Ultra-Resolution Optics

AWARD PERIOD: 1 July 1998 - 31 December 2000

REPORTING PERIOD: 1 July 1998 - 31 December 2000

OBJECTIVE: To design and produce new optical biosensors and methods for measuring analytes of importance to ONR with submicroscopic resolutions and high sensitivity.

APPROACH: Develop high resolution non-linear optical microscopy based on second harmonic generation. Detect single molecules via metallic nanoparticle enhancement of non-linear optical signals from styryl dyes. Exploit near infrared dye chemistry to develop new fluorescent transducers for biosensors.

ACCOMPLISHMENTS:

We have established Second Harmonic Imaging Microscopy (SHIM) as a new high resolution non-linear optical modality. This was achieved by preparing cells stained with several of our styryl dyes and imaging them with a laser scanning microscope coupled to a Ti-Sapphire laser capable of generating 100fs near infrared pulses. SHIM images could be directly compared to the images produced by 2-photon excitation. Because the SHG intensities are highly sensitive to membrane potential, SHIM shows great promise as a functional imaging modality for cell biology and may form a basis for cell-based physiological biosensors. As a serendipitous by product of this research, we have discovered that many intrinsic biological supramolecular assemblies can produce large second harmonic generation signals. These include skeletal muscle fibers, microtubule bundles and collagen in connective tissue. The ability to readily and non-destructively image these structures in 3 dimensions with sub-micrometer resolution and in living cells and tissue will lead to new dynamic imaging data that have been previously inaccessible.

We have extended the principle that roughened metal surfaces can produce enhancement of second harmonic generation (SHG) to investigate whether single gold nanoparticles can sensitize SHG from our styryl dyes. These styryl dyes were previously shown by us to be excellent substrates for SHG when they are bound to a monolayer or to one surface of a biological membrane. During this project we showed that gold nanoparticles on membrane surfaces that had been stained with the dyes could produce local enhancements of the SHG signal from the dye monolayers. Furthermore, these nanoparticles could be directed to

specific proteins on the surface and thus results in the ability to locate these molecules. Interestingly, the potential sensitivity of the SHG signal could be combined with this ability to locally enhance the SHG signal to enable the sensing of electrical activity on a molecular spatial scale.

Taking the idea of gold particle SHG enhancement one step further, we have constructed 100nm dye-gold conjugates where the gold particles are separated from the dye molecules by a thin 3nm polyacrylamide coating. SHG can be directly detected from individual dye-nanoparticle conjugates while no SHG is apparent from either bare gold nanoparticles or dye-latex nansphere conjugates. Futhermore, the nanoparticles can bind to a cell membrane and produce SHG that is at least a factor of 20 stronger than the dye by itself.

Both new styryl dye chromophores and technology for conjugating styryl dyes to proteins have been developed. We have completed the synthesis of 3 styryl dyes with appended maleimide groups and 2 additional dyes with iodoacetamide groups. These compounds can be used to conjugate cysteine groups in engineered protein receptors to construct new high sensitivity and high specificity biosensors. The dye labeling reagents developed thus far have absorbance around 500nm and emission around 650nm. They show remarkably high fluorescence enhancements upon conjugation compare to the unbound aqueous dye. In addition, we have developed a series of over a dozen new chromophores that have spectra further to the red with emissions as high as 900nm. The technology for appending iodoacetamide and maleimide moieties to these new dyes as well as conjugating protein sensors with currently available labeling reagents will be pursued. In addition to the ability of these dyes to give SHG signals, the styryl dyes have great advantages for conventional fluorescence applications because of their high environmental sensitivity and large Stokes shifts.

CONCLUSIONS: A new modality for non-linear optical microscopy, SHIM, has been established. Enhancement of SHG by gold nanoparticles has been demonstrated and shows great potential for single molecule sensing applications. A series of new dyes have been synthesized that have uniquely advantageous properties for both SHG and protein labeling applications.

SIGNIFICANCE: The unique ability to combine linear (i.e fluorescence) and non-linear (i.e. SHG and 2-photon excitation) optical phenomena in a single system of dyes and microscopic imaging devices promises unprecedented versatility and sensitivity in the analysis of materials of importance to the Navy such as transition metals in a marine environment. We have shown that second harmonic generation can be used for imaging and single molecule detection. This non-linear optical approach may develop into a non-destructive sensitive probe of 3D analyte distribution of relevance to the Navy. The new labeling reagents, because of the extraordinarily high sensitivity of their fluorescence to their environment, have great potential as the signal transducer elements in biosensor constructs. We have intitiated a collaboration with Dr. Homme Hellinga, also an ONR grantee, to explore the utility of the maleimide dyes as signal transducers in his engineered protein receptors. In addition, the non-linear optical and dye conjugate technologies are being transferred to a MURI project on stochastic sensing (Hagan Bayley, PI).

PATENT INFORMATION: Invention disclosure filed for new covalent labeling reagents.

AWARD INFORMATION: None

PUBLICATIONS AND ABSTRACTS (for total period of grant:

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